Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for generating a protein or peptide molecule, having a predetermined property or activity, the method comprising:

(a) identifying, within a target protein or peptide, one or more target amino acids amenable to providing the evolved predetermined property or activity upon amino acid replacement, wherein each target amino acid is designated an *in silico*-HIT (is-HIT);

(b) identifying one or more replacement amino acids, specific for each is-HIT, amenable to providing the evolved predetermined property or activity to the target protein upon amino acid replacement, wherein each single amino acid replacement within the target protein or peptide is designated as a candidate LEAD protein;

(c) producing a collection of sets of nucleic acid molecules that encode each of the candidate LEAD proteins, wherein:

each candidate LEAD protein contains a single amino acid replacement;

each nucleic acid in a set encodes the same candidate LEAD protein that differs by one amino acid from the target protein or [[peptide']] peptide;

each set is separate from all other sets;

(d) introducing each set of nucleic acid molecules into host cells and expressing the encoded candidate LEAD proteins, wherein the host cells are in an addressable array such that each lead protein is expressed at a different locus in the array;

(e) individually screening the sets of encoded candidate LEAD proteins to identify one or more proteins that has an activity that differs from an activity an unmodified target protein, wherein each such protein is designated a LEAD mutant protein.

Claim 2 (original): The method of claim 1, wherein the array comprises a solid support with separate loci and each set of cells is at a different locus.

Claim 3 (original): The method of claim 2, wherein the loci comprise wells; and each well contains one set of cells.

Claim 4 (original): The method of claim 1, wherein the nucleic acid molecules comprise plasmids; and the cells are eukaryotic cells that are transfected with the plasmids or are bacterial cells are transformed with the plasmids.

Claim 5 (original): The method of claim 1, wherein the nucleic acid molecules in step (c) are produced by site-specific mutagenesis.

Claim 6 (original): The method of claim 1, further comprising:

(f) generating a population of sets of nucleic acid molecules encoding a set of candidate super-LEAD proteins, wherein each candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins;

(g) introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells and expressing the encoded candidate super-LEAD proteins; and

(h) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

Claim 7 (original): The method of claim 6, wherein the nucleic acid molecules in step (f) are produced by a method selected from among Additive Directional Mutagenesis (ADM), multi-overlapped primer extensions, oligonucleotide-

mediated mutagenesis, nucleic acid shuffling, recombination, site-specific mutagenesis, and *de novo* synthesis.

Claim 8 (original): The method of claim 1 wherein the is-HITs identified in step (a) correspond to a restricted subset of amino acids along the full length target protein.

Claim 9 (original): The method of claim 1, wherein the replacement amino acids identified in step (b) correspond to a restricted subset of the 19 remaining non-native amino acids.

Claim 10 (original): The method of claim 1, wherein the nucleic acids of step (c) are produced by systematically replacing each codon that is an is-HIT, with one or more codons encoding a restricted subset of the remaining amino acids, to produce nucleic acid molecules each differing by at least one codon and encoding candidate LEADs.

Claim 11 (original): The method of claim 6, wherein the number of LEAD amino acid positions generated on a single nucleic acid molecule is selected from the group consisting of: two, three, four, five, six, seven, eight, nine, ten or more LEAD amino acid positions up to all of the LEAD amino acid positions.

Claim 12 (original): The method of claim 1, wherein the change in activity is at least about 10% of the activity of the unmodified target protein.
Claim 13 (original): The method of claim 1, wherein the change in activity is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

Claim 13 (original): The method of claim 1, wherein the change in activity is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

Claim 14 (original): The method of claim 1, wherein the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein.

Claim 15 (original): The method of claim 1, wherein the activity modified is selected from among increased catalytic activity, altered substrate and ligand recognition, increased thermostability, increased stability, increased resistance to proteases, increased resistance to glomerular filtration, increased immunogenicity, increased cationization, increased anionization and pseudo wild-type function.

Claim 16 (original): The method of claim 1, wherein each is-HIT target amino acid is susceptible to digestion by one or more proteases.

Claim 17 (original): The method of claim 16, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

Claim 18 (currently amended): The method of claim 1, wherein in a modified protein, each is-HIT target amino acid is resistant to digestion by one or more proteases compared to in [[unmodif]] <u>unmodified</u> protein.

Claim 19 (original): The method of claim 18, wherein the LEADs or super-LEADs possess increased digestibility compared to unmodified target protein.

Claim 20 (original): The method of claim 1, wherein each is-HIT target amino acid affects protein conformation and/or antigenicity.

Claim 21 (original): The method of claim 20, wherein the LEADs or super-LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

Claim 22 (original): The method of claim 1, wherein each is-HIT target amino acid affects protein amphipathic properties.

Claim 23 (original): The method of claim 22, wherein the LEADs or super-LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

Claim 24 (original): The method of claim 1, wherein each is-HIT target amino acid is amenable to constitute a link or bridge between two regions of a protein.

Claim 25 (original): The method of claim 24, wherein the LEADs or super-LEADs possess increased thermostability compared to unmodified target protein.

Claim 26 (original): The method of claim 1, wherein each is-HIT target amino acid affects binding affinity to its cognate receptor.

Claim 27 (currently amended): The method of claim 26, wherein the LEADs or super-LEADs possess either increased or decreased binding affinity to [[its]]<u>the LEADs or super-LEADs</u> cognate receptor compared to unmodified target protein.

Claim 28 (currently amended): A method for generating proteins with a desired property, comprising:

(a) identifying a target protein;

(b) identifying is-HIT target residues associated with the property;

(c) preparing a collection of variant nucleic acid molecules encoding a collection of variant proteins, wherein each variant nucleic acid encodes a candidate LEAD mutant protein that differs by one replacement amino acid from the target protein at one is-HIT target residue;

(d) separately introducing the nucleic acids encoding each candidate LEAD protein into hosts for expression thereof and expressing the nucleic acid molecules encoding each variant protein;

(e) screening each variant LEAD candidate [[proteins]] <u>protein</u> to identify any that have an activity that differs by a predetermined amount from the activity of the unmodified target protein, thereby identifying proteins that are LEADs.

Claim 29 (original): The method of claim 28, wherein either: each of the identified is-HIT target residues in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids; or the total number of is-HIT residues that are replaced with replacement amino acids is less than the total amount of amino acid residues within the full-length of the target protein.

Claim 30 (original): The method of claim 28, wherein each of the identified is-HIT residues in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids.

Claim 31 (original): The method of claim 28, wherein the total number of is-HIT residues that are replaced with replacement amino acids is less than the total amount of amino acid residues within the full-length of the target protein.

Claim 32 (original): The method of claim 28, wherein each of the identified is-HIT residues in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids; and the total number of is-HIT residues that are replaced with replacement amino acids is less than the total amount of amino acid residues within the full-length of the target protein.

Claim 33 (currently amended): The method of claim 28, further comprising:

[[(d)]] (f) generating a population of sets of nucleic acid molecules encoding a set of candidate super-LEAD proteins, wherein each candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins;

[[(e)]] (g) introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells and expressing the encoded candidate super-LEAD proteins; and

[[(f)] (h) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

Claim 34 (currently amended): The method of claim 33, wherein the nucleic acid molecules in step [[(f)]] (h) are produced by a method selected from among additive directional mutagenesis (ADM), multi-overlapped primer extensions, oligonucleotide-mediated mutagenesis, nucleic acid shuffling, recombination, site-specific mutagenesis, and *de novo* synthesis.

Claim 35 (original): The method of claim 33, wherein the number of LEAD amino acid positions generated on a single nucleic acid molecule is selected from the group consisting of: two, three, four, five, six, seven, eight, nine, ten or more LEAD amino acid positions up to all of the LEAD amino acid positions.

Claim 36 (original): The method of claim 28, wherein each is-HIT target residue is susceptible to digestion by one or more proteases.

Claim 37 (original): The method of claim 36, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

Claim 38 (original): The method of claim 28, wherein each is-HIT target residue is resistant to digestion by one or more proteases.

Claim 39 (original): The method of claim 38, wherein the LEADs or super-LEADs possess increased digestibility compared to unmodified target protein.

Claim 40 (original): The method of claim 28, wherein each is-HIT target residue affects protein conformation.

Claim 41 (original): The method of claim 40, wherein the LEADs or super-LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

Claim 42 (original): The method of claim 28, wherein each is-HIT target amino acid affects protein amphipathic properties.

Claim 43 (original): The method of claim 42, wherein the LEADs or super-LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

Claim 44 (original): The method of claim 28, wherein each is-HIT target amino acid is amenable to constitute a link or bridge between two regions of a protein.

Claim 45 (original): The method of claim 44, wherein the LEADs or super-LEADs possess increased thermostability compared to unmodified target protein.

Claim 46 (original): The method of claim 28, wherein each is-HIT target amino acid affects binding affinity to its cognate receptor.

Claim 47 (currently amended): The method of claim 46, wherein the LEADs or super-LEADs possess either increased or decreased binding affinity to [[its]]the LEADs or super-LEADs cognate receptor compared to unmodified target protein.

Claim 48 (original): The method of claim 28, wherein the change in activity is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

Claim 49 (currently amended): The method of claim 28, wherein the change [[inactivity]] in activity is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

Claim 50 (original): The method of claim 28, wherein the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein.

Claim 51 (original): A method for the production of a protein having an evolved property or activity compared to a unmodified target protein, the method comprising:

(a) selecting, on the target protein, one or more target amino acids amenable to providing the evolved property or activity upon amino acid replacement;

(b) replacing each target amino acid with a replacement amino acid amenable to providing the evolved property or activity to form a candidate LEAD protein, wherein only one amino acid replacement occurs on each target protein;

(c) expressing from a nucleic acid molecule each candidate LEAD protein in a cell contained in an addressable array; and

(d) assaying each candidate LEAD protein for the presence or absence of the evolved property or activity compared to a unmodified target protein, thereby identifying proteins that are LEADs.

Claim 52 (original): The method of claim 51, wherein the selection of the one or more target amino acids in step a) is conducted *in silico* and the targets amino acids are designated is-Hits.

Claim 53 (original): The method of claim 52, wherein the *in silico* selection step further comprises selecting an is-HIT target residue that is susceptible to digestion by one or more proteases.

Claim 54 (original): The method of claim 53, wherein the LEADs possess increased resistance to proteolysis compared to unmodified target protein.

Claim 55 (currently amended): The method of claim 52, wherein the *in silico* selection step further comprises selecting an is-HIT target residue <u>that</u> is resistant to digestion by one or more proteases.

Claim 56 (original): The method of claim 55, wherein the LEADs possess increased digestibility compared to unmodified target protein.

Claim 57 (currently amended): The method of claim 52, wherein the *in silico* selection step further comprises selecting an is-HIT target residue that affects protein conformation and/or immunogenicity.

Claim 58 (original): The method of claim 57, wherein the LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

Claim 59 (currently amended): The method of claim [[51]] 52, wherein the *in silico* selection step further comprises selecting an is-HIT target amino acid affects protein amphipathic properties.

Claim 60 (original): The method of claim 59, wherein the LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

Claim 61 (currently amended): The method of claim 60, wherein the *in silico* selection step further comprises selecting an is-HIT target amino acid that is amenable to constitute a link or bridge between two regions of a protein.

Claim 62 (original): The method of claim 61, wherein the LEADs possess increased thermostability compared to unmodified target protein.

Claim 63 (currently amended): The method of claim 62, wherein the *in silico* selection step further comprises selecting an is-HIT target amino acid that affects binding affinity to its cognate receptor.

Claim 64 (original): The method of claim 63, wherein the LEADs possess either increased or decreased binding affinity to its cognate receptor compared to unmodified target protein.

Claim 65 (original): The method of claim 51, wherein the change in activity is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

Claim 66 (currently amended): The method of claim 51, wherein the change [[inactivity]] <u>in activity</u> is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

Claim 67 (original): The method of claim 51, wherein the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein.

Claim 68 (original): A method of displaying the amino acid sequence of a protein, said method comprising:

providing a first axis that corresponds to amino acid positions along the length of the protein sequence, wherein each amino acid position is designated as a position-cell;

providing a second axis at each amino acid position within said protein, wherein said second axis contains 20 type-cells thereon, wherein each type-cell corresponds to a mutually exclusive amino acid; and

indicating the particular amino acid residue at the respective cell-type/position-cell intersection by a detectable signal.

Claim 69 (original): The method of claim 68, wherein the number of position-cells is variable depending on the size of the protein.

Claim 70 (original): The method of claim 68, wherein the number of position-cells equals the number of amino acids in the protein sequence.

Claim 71 (original): The method of claim 68, wherein the first axis is vertical and the second axis is horizontal.

Claim 72 (original): A medium, comprising a matrix display produced by the method of claim 68.

Claim 73 (original): The medium of claim 72 that is can be read visually or that is computer readable.

Claim 74 (currently amended): A two-dimensional (2-D) matrix representation of a protein sequence comprising:

a first axis that corresponds to amino acid positions along the length of the protein sequence, wherein each amino acid position is designated as a position-cell;

a second axis at each amino acid position within said protein, wherein said second axis contains 20 type-cells thereon, wherein each type-cell corresponds to a mutually exclusive amino acid; and

an ~~indentifier~~ identifier indicating the particular amino acid residue at the respective cell-type/position-cell intersection.

Claim 75 (original): A method for making a modified protein having substantially the same activity as unmodified protein, the method comprising:

replacing each amino acid position over the entire length of a target protein with the same reference amino acid, wherein only one reference amino acid is substituted on each molecule, to form a candidate HIT;

assaying each candidate HIT for a change in a selected protein activity;

identifying each locus on the target protein that is amenable to amino acid replacement without change in the protein activity as a pseudo-wild type position.

Claim 76 (original): The method of claim 75, further comprising replacing one or more pseudo-wild type positions with candidate pseudo-wild type amino acids, wherein an amino acid replacement that does not result in a decrease in the requisite protein activity is designated a pseudo-wild type amino acid at that pseudo-wild type position.

Claim 77 (original): The method of claim 76, wherein the change in a protein activity is a decrease.

Claim 78 (original): The method of claim 76, wherein at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, of amino acid residue positions on a target protein are replaced.

Claim 79 (currently amended): The method of claim 1, wherein the [[replacing]] <u>replacement</u> amino acids are selected using Percent Accepted Mutations (PAM) matrices.

Claim 80 (currently amended): The method of claim 1, wherein the [[replacing]] <u>replacement</u> amino acids are pseudo-wild type amino acids.